Stereoselective Reduction of Radicinin by Liquid Cultures of Alternaria longipes

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STEREOSELECTIVE REDUCTION OF RADICININ BY LIQUID CULTURES OF *ALTERNARIA LONGIPES*

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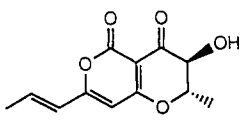
ABSTRACT.—Cultures of *Alternaria chrysanthemi* normally produce the metabolites radicinin [**1**] and radicinol [**2**]. An antagonistic interaction was observed when *A. chrysanthemi* and *Alternaria longipes* were grown in dual plate and liquid culture. The sole product recovered from such cultures was 3-*epi*-radicinol [**3**]. In subsequent experiments cultures of *A. longipes* were shown to biotransform **1** to **2** and **3**. The stereoselectivity observed in this biotransformation appears to be dependent on the nature of the fungal growth medium.

Plant pathogenic fungi belonging to the genus *Alternaria* (Dematiaceae) are of worldwide distribution and great economic importance. The most prevalent diseases of *Alternaria* include leaf spot, blight, fruit spot, and stem canker. Host plants include tobacco, sunflower, tomato, apple, pear, and citrus (1).

As part of our ongoing search to find a successful method for the biological control of members of the genus *Alternaria*, a series of *Alternaria* strains were grown in paired plate culture. This technique is used to observe antagonistic interactions between fungal species and to stimulate secondary metabolite production (2,3). Of the test organisms examined, an antagonism was observed when *Alternaria longipes* (Ellis and Everh.) Mason and *Alternaria chrysanthemi* Simmons and Crosier were grown in paired plate culture on malt extract agar. The antagonism that was observed took the form of an inhibition of fungal growth along the line of mycelial contact. The agar from the mycelial contact zone was extracted with EtOAc, and the residue was purified to yield a single compound which was identified as 3-*epi*-radicinol [**3**] by analysis of its spectral data and by comparison with literature values for synthetic **3** (4). Short term dual liquid cultures (grown for 10 days on malt extract broth) also produced **3** as the sole product. This is the first report of **3** as a natural product.

Liquid cultures of *A. chrysanthemi* have been shown to produce **1** and **2** as normal metabolites with the concentration of **2** only reaching a quantifiable level after 10 days of growth (5,6). When *A. chrysanthemi* was antagonized in paired plate culture with the fungus *Phytopthora erythroseptica*, the only compound produced was radicinin [**1**] (2). The sole metabolite reported to date from *A. longipes* cultures is the tetramic acid tenuazonic acid [**4**] (7).

The results of the paired culture experiments suggest that a rapid selective reduction of **1** to **3**, catalyzed by *A. longipes*, is occurring in dual culture. In order to confirm this proposal, **1** (obtained from liquid cultures of *A. chrysan-*



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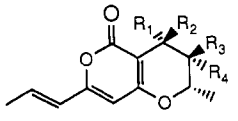
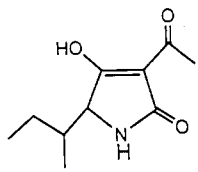


2 $R_1=R_3=OH$, $R_2=R_4=H$
3 $R_1=R_4=H$, $R_2=R_3=OH$



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themi), was incubated with cultures of *A. longipes* grown on malt extract broth, and the time course for the biotransformation of **1** to **3** was monitored. Hplc analysis indicated the disappearance of **1** from the growth medium in association with the appearance of **3**. The total biotransformation of **1** to **3** occurred within a 5-day period. The biotransformation catalyzed by *A. longipes* yielded only 3-*epi*-radicinol [**3**], which differs in physical and spectroscopic properties from its diastereoisomer **2** (4).

When the *A. longipes*-catalyzed biotransformation of **1** was carried out in potato dextrose broth, the results observed were quite different. At the end of a 5-day incubation period the recovered product was shown, by detailed nmr analysis, to be a 2:1 mixture of **2** and **3**. The products were separable by cc on Si gel using a $CHCl_3/MeOH/H_2O$ gradient. The relative concentrations of **2** and **3** in the biotransformation mixture could not be quantified by hplc due to the small difference in their retention times. However, the diastereomeric excess of **2** (44%) could be measured by integration of the signals for the H-3 protons of the diastereoisomers which resonate as doublets at 4.70 ppm ($J = 6.5$ Hz) for **2** and 4.78 ppm ($J = 4$ Hz) for **3** (Figure 1).

From these results we conclude that *A. longipes* produces a stereoselective reductase enzyme which can reduce radicinin [**1**] to a diol.

The stereospecificity of the enzymic reduction may depend on growth conditions, which would be in keeping with the observation that carbon source can influence the enzymes produced by a microorganism and this can in turn lead to a difference in the stereochemical course of the reduction (8). The identification of a stereoselective reductase enzyme in *A. longipes* cultures is also of potential economic importance since there is growing interest in the utilization of enzymes in the production of chiral molecules for the pharmaceutical and agrochemical industries (9).

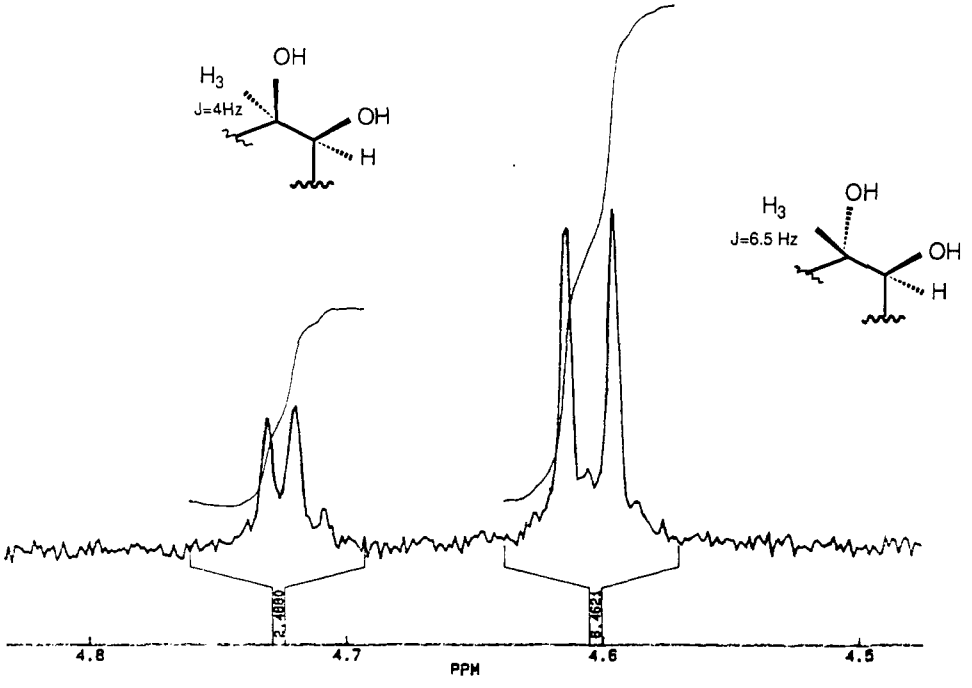

FIGURE 1. Coupling constants for radicinol [**2**] and 3-*epi*-radicinol [**3**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr spectra were recorded at 400 MHz on a Bruker AC-400 spectrometer, using $CDCl_3$ as solvent with TMS as internal standard. Mass spectra were recorded in the ci mode at 70 eV. Analytical tlc was carried out on plastic sheets pre-coated (0.2 mm) with Si gel $60F_{254}$ (E. Merck Darmstadt). Compounds were detected by visualization at uv 254 nm. Hplc was carried out on a Kontron hplc system 600 equipped with a uv detector (310 nm). A normal phase column packing was used [mobile phase $CHCl_3$-MeOH (98:2), flow rate 4 ml/min; column dimensions 15 cm × 4.6 mm].

DUAL FUNGAL CULTURES.—Malt agar plates were inoculated at opposing sides of plates with 4-mm cores of *A. chrysanthemi* (CBS 421.65) and *A. longipes* (CBS 113.35). Plates were incubated for 10 days in the dark at 25°. Dual broth cultures of *A. chrysanthemi* and *A. longipes* were prepared by inoculating 150 ml malt extract broth (in 250-ml conical flasks) with 4 plugs (each 4 mm in diameter) of each fungus taken from the growing edge of a culture grown on malt extract agar. Cultures were agitated on an orbital shaker at 90 rpm and grown at 25° (±2°) in a light (18 h)/dark (6 h) cycle.

EXTRACTION OF PRODUCTS.—Cultures were filtered, and fungal growth medium was extracted with EtOAc. This was dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The resulting residue was chromatographed on a Si gel column using a $CHCl_3$/MeOH gradient as eluent.

BIOTRANSFORMATION OF RADICININ [**1**].—Conical flasks (250 ml) containing malt extract broth (150 ml) or potato dextrose broth (150 ml) were inoculated with cores of *A. longipes* (4 × 4 mm) grown on the appropriate agar. Cultures were incubated on an orbital shaker for 5 days. Flasks were then inoculated with an MeOH solution (5 ml) of **1** at a final concentration of 1 μg of **1** per ml of culture broth. Substrate controls which contained **1** and medium with no cells at the same final concentration were also set up. Flasks, in triplicate, were harvested every 24 h for 5 days, the relative percentage concentration of products was determined by hplc [R_s for **1** and **3** = 2.75 (SD 0.37)]. The products were isolated and purified as outlined. Compounds were examined by tlc using $CHCl_3$-MeOH-H_2O (10:1:0.1), and were characterized by nmr.

COMPOUNDS ISOLATED.—*Radicinin* [**1**].—Compound **1** was isolated from *A. chrysanthemi* cultures grown on Czapek Dox broth: mp 219° [lit. (6) mp 235–238°]; $C_{12}H_{12}O_5$ ($[M]^+$ 236.0684); R_f 0.32; hplc Rt 4.30 min.

Radicinol [**2**].—Compound **2** was isolated as an oil from *A. chrysanthemi* cultures grown on Czapek Dox broth: $[\alpha]_D$ −168 (c = 1, $CHCl_3$); $C_{12}H_{14}O_5$ ($[M]^+$ 238.0841); R_f 0.16; hplc Rt 4.85 min.

3-epi-Radicinol [**3**].—Compound **3** was isolated as an oil: $C_{12}H_{14}O_5$ ($[M]^+$ 238.0842); $[\alpha]_D$ −100 (c = 1, MeOH), −84° (c = 1, $CHCl_3$) [lit. (4) −92° (c = 0.48, $CHCl_3$)]; R_f 0.14; hplc Rt 5.45 min; ^{1}H nmr 1.49 (3H, d, J = 6.8, Me-12), 1.90 (3H, dd, J = 5.9, 1.3, Me-11), 3.44 (1H, s, OH), 3.68 (1H, dd, J = 8.8, 4.0, H-4), 4.20 (1H, dq, J = 8.8, 6.8, H-5), 4.30 (1H, bs, OH), 4.63 (1H, d, J = 4, H-3), 5.78 (1H, s, H-8), 5.96 (1H, d, J = 13, H-9), 6.68 (1H, dq, J = 13, 5.9, 1.2, H-10).

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LITERATURE CITED

1. G.N. Agrios, "Plant Pathology," 2nd ed., Academic Press, New York, 1978, pp. 316–319.
2. W. Newton, *Can. J. Bot.*, **31**, 423 (1953).
3. D.M.X. Donnelly and H. Sheridan, *Phytochemistry*, **10**, 2303 (1986).
4. M. Nukina and S. Marumo, *Tetrahedron*, **37**, 3271 (1977).
5. D.J. Robeson, G.R. Gray, and G.A. Strobel, *Phytochemistry*, **21**, 2359 (1982).
6. J.F. Grove, *J. Chem. Soc.*, 3234 (1964).
7. C.E. Stickings and R.J. Townsend, *Biochem. J.*, **78**, 412 (1961).
8. A. Fauve and H. Veschambre, *Biocatalysis*, **3**, 95 (1990).
9. N.J. Turner, *Nat. Prod. Rep.*, **6**, 625 (1989), and references cited therein.

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